



IN THE UNITED STATES PATENT AND TRADE MARK OFFICE

**Applicant:** Levon Michael Khachigian, Ph.D.

**Assignee:** Unisearch Limited

**Serial No:** 09/757, 555

**Filed:** 9 January 2001

**Title:** Inhibition of Proliferation of Cells

**Examiner:** Janet L Epps, Ph.D.

**Group Art Unit:** 1635

---

DECLARATION UNDER 37 CFR §1.132

I, LEVON MICHAEL KHACHIGIAN, declare and say as follows:

1. I received my PhD in molecular biology from the University of New South Wales (UNSW, Sydney) in 1993. I have done post-doctoral work at the Department of Pathology, Brigham and Women's Hospital & Harvard Medical School from 1993-1995. I currently hold the position of Associate Professor of Pathology (UNSW) and Principal Research Fellow of the National Health and Medical Research Council of Australia (NHMRC). A copy of my curriculum vitae, including a list of publications for which I am author or coauthor is annexed hereto and marked "Annexure A".
2. I am the inventor of patent application serial No. 09/757, 555.
3. I have read and am thoroughly familiar with patent application serial No. 09/757, 555. Further, I have read and am thoroughly familiar with the office action dated 23 April 2002. I am making this declaration in support of the pending claims of patent application serial No. 09/757, 555.
4. In the office action dated 23 April 2002, the Examiner has rejected claims 1 and 2 under 35 USC § 102 (b) as being anticipated by Hu *et al* (1994). The Examiner contends that the Hu *et al* document "teaches the regulation of Tis-8 (ie. the rat homologue of Egr-1) in a rat glioma cell line (page 1825, paragraph 3 and Figure 7 of the document)". The Examiner correctly points out that glioma cells were used in the Hu *et al* (1994) paper (Figure 7). However, Figure 7 merely shows that Tis 8 is expressed in glioma cells. Unlike the

preceding figures showing the capacity of atrial natriuretic peptide (ANP) or endothelin (ET-3) to modulate Tis-8 expression in astrocytes, Figure 7 demonstrates "importantly, (that) both ANP and ET-3 failed to inhibit or stimulate, respectively, the expression of this gene" (page 1825, paragraph 3, lines 8-10). This is taught again in the Figure 7 legend ("there was no effect of either ANP or ET on Tis 8 expression in these cells", page 1825), and in the Discussion ("our findings in glioma cells indicate that ANP can not inhibit and endothelin can not stimulate the basal high expression of this gene", page 1826, paragraph 4, lines 1-3). This disparity in Tis 8 responsiveness to ANP or ET-3 in astrocytes and glioma cells was noted, as the authors indicate, despite both these cells having being "well characterized as having both ANP and ET cell surface receptors" by other groups, including the authors themselves (page 1825, paragraph 3, lines 2-5). The authors speculate that the mechanism controlling Tis-8 expression in cultured glia/glioma cells "is lacking" in astrocytes (page 1826, paragraph 4, lines 3-4). Therefore, the Hu *et al* (1994) document, clearly does not teach compounds capable of regulating or specifically inhibiting Tis-8 expression or activity in glioma cells.

5. The claims of US 09/757555 are directed to a **method of screening for compounds** which can inhibit proliferation of vascular and neoplasia cells, wherein the compounds are selected by their ability to inhibit Egr-1 expression or activity. In contrast, Hu *et al* (1994) is focussed on deducing the intracellular pathway by which ANP and ET-3 regulate astrocyte proliferation. It is not concerned with methods to screen for compounds which can inhibit proliferation of vascular and neoplasia cells. Astrocytes are not a type of vascular or neoplastic cell. Moreover, Hu *et al* (1994) did not find that Tis-8 expression was altered by ANP and ET-3 in glioma cells (as discussed in 4 above) and did not use antisense Tis-8 with glioma cells.

6. In the office action dated 23 April 2002, the Examiner rejected claims 1 to 3 under 35 USC § 103(a) on the basis that the claimed invention is obvious in view of Mendelsohn *et al* (1994). I have reviewed Mendelsohn *et al* (1994) and consider that the document claims that agents which (i) **inhibit** vascular smooth muscle cell activation and/or proliferation; (ii) **enhance** vascular endothelial cell activation and/or proliferation; or (iii) **activate** estrogen responsive genes in vascular cells, are useful as putative therapeutic agents for cardiovascular disease (see column 1, lines 43 to 49). The document teaches that methods for screening for vasoprotective agents can include (i) examining the effect of the candidate agent on cell activation and/or proliferation; or **independently** (ii) examining the effect of a candidate agent on the expression of an estrogen responsive gene.

7. The Examiner points out that Mendelsohn *et al* (1994) provides screening methods that can be used to identify vasoprotective agents which inhibit vascular smooth muscle cell activation and/or proliferation, enhance vascular endothelial cell activation and/or

proliferation, or activate estrogen responsive genes in vascular cells. The Examiner concludes that the document teaches methods of identifying vasoprotective agents by their ability to influence the expression of an estrogen responsive gene. However, as the Examiner correctly indicates (Office Action, page 4, paragraph 2), the document does not specifically describe a method of screening for compounds that inhibit proliferation of cells based on their ability to inhibit Egr-1.

8. The Examiner states that the cited document asserts the preferred effect of the potential vasoprotective agent on the expression of Egr-1 is to decrease (-/-) the expression of Egr-1 in vascular smooth muscle cells and vascular endothelial cells (column 11, lines 46 to 48, and line 54). However, this directly contradicts the main teaching of the document that it is desirable to activate estrogen responsive genes in vascular cells (column 1, lines 43 to 49, column 1, line 64 to column 2, line 7, column 12, lines 25-27). Egr-1 is claimed to be an estrogen responsive gene (column 11, lines 22 to 29), yet it is explicitly stated that the preferred agent would inhibit (-/-) Egr-1 expression in vascular endothelial cells and vascular smooth muscle cells. Therefore, inhibiting Egr-1 activity strongly contradicts the teachings of the document. Since the document teaches that estrogen has an atheroprotective function (column 1, paragraph 5), and claims that estrogen induces Egr-1, it is simply not consistent to claim that the preferred agent would suppress (-/-) Egr-1 in vascular smooth muscle cells and endothelial cells.

9. The document asserts that the preferred agent would inhibit vascular smooth muscle cell activation/proliferation or stimulate endothelial cell activation/proliferation. However, there is no molecular or cellular rationale whatsoever provided in the document why the expression of Egr-1, or indeed that of 19 other genes (listed in column 11, paragraph 6), should be increased or decreased by the preferred agent, beyond mere responsiveness to estrogen. Moreover, there is no primary data, published during or before 1994, of which I am aware, that estrogen can even induce Egr-1 expression in vascular smooth muscle cells or endothelial cells.

10. In the list of genes whose expression would be increased or decreased by the preferred agent (see column 11), there is no sound rationale for the desired outcomes. For example, in vascular endothelial cells, the authors assert that the preferred agent would inhibit (-) Egr-1 expression, but stimulate (+) c-Fos expression, whereas in vascular smooth muscle cells, the preferred agent would inhibit both factors. Egr-1 and c-Fos are both immediate-early genes and nuclear transcription factors, claimed to be induced by estrogen, that switch on the expression of mitogenic genes, the latter via AP1. It is puzzling why a given agent would be desired to inhibit one transcription factor yet stimulate the other.

11. In view of the contradictions discussed in the preceding paragraphs, I do not consider that the Mendelsohn *et al* (1994) document provides any clear guidance to a person of ordinary skill in the field of cell biology to achieve the invention defined in the claims of US 09/757555.
12. There is no clear suggestion in Mendelsohn *et al* (1994) of a method of screening for compounds which can inhibit proliferation of vascular cells and neoplasia cells. In fact, neoplasia cells are not discussed in the document at all. Claim 2 of US 09/757555 requires that the method is suitable for inhibiting proliferation specifically of neoplasia cells. Moreover, with regard to pending claim 3, the invention requires that the method is suitable for inhibiting both vascular smooth muscle cells **and** inhibiting vascular endothelial cells. Notwithstanding the above, Mendelsohn *et al.* (1994) teaches that inhibition of the proliferation of vascular smooth muscle cells but enhancement of the proliferation of vascular endothelial cells are required (see column 1, lines 51 to 63).
13. US 09/757555 clearly teaches and demonstrates that compounds which can inhibit proliferation of vascular (smooth muscle cells and endothelial cells) and neoplasia cells can be selected by their ability to inhibit Egr-1 expression or activity. Therefore, for the reasons discussed above, I do not consider that any of the documents cited by the Examiner provide clear directions to a person of ordinary skill in the field of cell biology to achieve the invention of the pending claims of US 09/757555.
14. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that wilful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of the Title 18 of the United States Code, and that such wilful false statements may jeopardise the validity of this application or any patent issuing thereon.

Dated: October 16, 2002



Signed: LEVON MICHAEL KHACHIGIAN